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Optimal fermentation time and temperature to improve biochemical composition and sensory characteristics of black tea

Moazzam Hassanpour Asil[1*], Babak Rabiei[2], Reza Hojjat Ansari[1]

[1]Department of Horticultural Sciences, Faculty of Agricultural Sciences, University of Guilan, P.O. Box 41635-1314, Rasht, Iran
[2]Department of Agronomy and Plant Breeding, Faculty of Agricultural Sciences, University of Guilan, P.O. Box 41635-1314, Rasht, Iran

***Corresponding author: hassanpurm@yahoo.com; hassanpurm@guilan.ac.ir**

Abstract

The effects of variations in fermentation time and temperature on the changes in theaflavins (TF), thearubigins (TR) as well as sensory characteristics of color liquor brightness, briskness and astringency of black tea were investigated in two cultivars; Promising 100 and Chinese. The two independent variables investigated in this experiment were fermentation duration at four levels (30, 60, 90 and 120 min) and fermentation temperature at three levels (20, 25 and 30°C).The results indicated that TR content and total color in black tea varied with cultivars showing higher levels in cultivar "Promising 100" than in cultivar "Chinese", while TF, brightness and organoleptic properties did not differ in the two cultivars. The processing of black tea at low fermentation temperatures improved black tea quality, because low fermentation temperatures caused higher TF levels. Moreover, higher levels of TF can also be produced with reduction in fermentation time. Positive correlation was seen between TF levels and total color, taster's briskness and astringent taste (r= 0.385, 0.863 and 0.407, respectively, P< 0.01) while TR levels showed positive correlation with color (r= 0.704, P< 0.01) and negative correlation with spectrophotometer brightness and taster's brightness (r= -0.401, P< 0.01).

Keywords: Astringent, Brightness, Briskness, Theaflavin, Thearubigin.
Abbreviations: TF- Theaflavin; TR- Thearubigin; CTC- Crush, Tear- Curl.

Introduction

Black tea is consumed throughout the world for its unique taste, briskness and flavor. Tea is the most widely consumed and cheapest non-alcoholic drink like next to water. Black tea is manufactured from the tender leaves of *Camellia sinensis* (L.) O. Kuntze (Muthumani and Kumar, 2007). Catechins are the major biochemical constituents (amounting to ca. 20% on dry weight basis) present in tea leaves and they get oxidized to form theaflavins (TFs) and thearubigins (TR) during fermentation (Obanda et al., 2001; Obanda et al., 2004; Yao et al., 2006; Muthumani and Kumar, 2007; Hsiao et al., 2010). Catechins and their oxidation products are mainly responsible for the taste and astringent character of black tea (Liang et al., 2003; Saravanan et al., 2005; Muthumani and Kumar, 2007). Apart from quality characters, catechins are also found to possess properties of benefit to human health (Muthumani and Kumar, 2007). TF, TR and theabrownins are complex phenolic compounds derived from the oxidation of catechins and their gallates during the fermentation stage of black tea processing (Yao et al., 2006). TF contributes the brisk and astringent taste and bright golden color to black tea quality, while TR contribute the reddish color and richness in taste, totally termed 'body' to black tea (Gulati et al., 1999; Obanda et al., 2001; Obanda et al., 2004; Yao et al., 2006). Although contribution of TR as well as that of TF to quality is acknowledged, the relationship between TR levels and quality remains obscure (Owuor et al., 2006). TR is acidic brown pigments formed by the oxidative degradation of TF. TR is polymeric proanthocyanidins, and it is reported that some TR are derived from theaflavic acids during fermentation (Obanda et al., 2004). The time, temperature, pH, relative humidity and oxygen availability during fermentation are the crucial factors responsible for the formation of high levels of desired products. Of these, duration of fermentation is important, since both increase and decrease in fermentation time can lead to poor quality tea (Owuor and Obanda, 2001; Muthumani and Kumar, 2007). For the processing of high quality black tea, it is critical to determine and use correct fermentation time. Consequently, different methods have been evaluated to assess optimal fermentation time. These efforts have concentrated on monitoring the formation of some black tea quality parameters (Owuor and Obanda, 2001). Studies on the effects of processing parameters on black tea chemical and sensory quality have demonstrated a decline in the levels of total TF, liquor brightness and briskness, with extended fermentation time and rise in temperature (Obanda et al., 2001). Organoleptical characteristics of black tea are assessed, using either sight, smell, and/or taste of the beverage. Previous studies have demonstrated linkages between the sensory quality characteristics of resultant black tea, the chemical composition of the green leaf and the black tea processing parameters (Obanda et al., 2001). This study was done to assess whether there are interactions between the effects of fermentation duration and temperature, and whether the two varieties (Promising 100 and Chinese) react in a similar way to variations in fermentation duration and temperature. Also, the responses of TF and TR, as well as sensory changes in liquor color, brightness, briskness and astringency to variations in fermentation time and temperature were investigated.

Results

The variations in black tea chemical and sensory quality parameters as a result of fermentation duration and temperature are presented in Table 1. Temperature variations had significant effects on the TF and TR content ($P \leq 0.01$), but not on other biochemical parameters. Fermentation duration had significant effects ($P \leq 0.05$) only on the TF content. Significant effects of cultivar on TR content ($P \leq 0.01$) and total color ($P \leq 0.05$) were observed. The effects of cultivar, fermentation duration and temperature on sensory parameters in black tea are presented in table 2. There were significant interactions ($P \leq 0.05$) in the effects of cultivar and replication on the astringent taste of black tea liquor. Effects of fermentation temperature were significant only for briskness and astringent taste ($P \leq 0.05$). There were significant interactions ($P \leq 0.01$) in effects of cultivar and fermentation duration or fermentation temperature on the brightness of black tea liquor ranking by taster (Table 2). The variation in black tea chemical and sensory quality parameters due to cultivar, fermentation duration and temperature are given in Tables 3 to 6. The quantification of the TR content and total color in the two tea cultivars is presented in Tables 3 and 4. Cultivar "Promising 100" produced high level of TR and more color compared to cultivar "Chinese". With increase in fermentation duration, liquor brightness scores evaluated by tasters declined in the cultivar "Promising 100",but in the cultivar "Chinese", it increased to a maximum and then slightly declined as fermentation duration further increased (Table 3). However, there were variations in the taster's evaluation of brightness of tea liquors and highest scores were reached at different fermentation times for the two cultivars. The TF and sensory evaluation levels (briskness and astringent taste) increased at 25 °C, and then decreased with increase in fermentation temperature at 30 °C, while the TR levels increased with increasing fermentation temperature (Table 5). Also, increasing fermentation duration from 30 to 120 min led to significant decreases in TF levels (Table 5). However, significant interaction was not seen in the effects of cultivar, fermentation temperature and duration on the formation of TF (Table 6). Correlation analysis was carried out with the TF and TR content against the spectrophometer brightness and total color, and taster's briskness, brightness and astringent taste of black tea are presented in Table 7. A positive correlation was observed between spectrophotometer total color and TF and TR content of black tea ($r = 0.385$ and 0.704, respectively, $P \leq 0.01$). However a negative correlation was detected between total color and spectrophotometer brightness and taster's brightness ($r = -0.498$ in the both parameters, $P \leq 0.01$) with total color. Taster's briskness showed a positive correlation ($r = 0.331$, $P \leq 0.01$). The spectrophotometer brightness had a negative correlation with TR content ($r = -0.401$, $P \leq 0.01$). TF levels had a positive correlation with taster's briskness ($r = 0.863$, $P \leq 0.01$). Also, the taster's briskness had a positive correlation with taster's astringent taste ($r = 0.417$, $P \leq 0.01$). The taster's brightness had a negative correlation with TR levels ($r = -0.401$, $P \leq 0.01$). The TF levels had a positive correlation ($r = 0.407$, $P \leq 0.01$) with the taster's astringent taste. The TF levels did not appear to be contributors to either test of brightness as the coefficients achieved were only -0.193 for both taster's brightness and spectrophotometer brightness (Table 7).

Discussion

The TR content and therefore, total color was significantly higher in the cultivar "Promising 100" than in "Chinese" cultivar. Similar results were reported by Owuor et al. (1987) and Owuor and Obanda (2001). However, the chemicals responsible for the taste and appearance of black tea are TF and TR. TF is responsible for the astringency, brightness, orange color and briskness of the black tea, while TR contribute to the mouth feeling (thickness) and brownish color of the tea (Wang et al., 2000; Owuor and Obanda, 2001). Thus, higher TR levels in cultivar "Promising 100" caused higher color levels in the black tea liquor. The cultivar "Promising 100" showed significant interactions with fermentation duration for taster's brightness. Black tea liquor was significantly brighter when fermentation time was increased to 60 min in the cultivar "Promising 100". These results demonstrated that some cultivars can be corrected for quality parameters, for example brightness by varying fermentation time. According to previous studies (Owuor and Obanda, 2007), biochemical parameters which enhanced black tea quality, are shown by high amounts of TF and brightness and lower levels of TR. It also enhanced sensory quality of black tea. Thus, to improve the quality of cultivar "Promising 100", it should be fermented at shorter duration (30 min). The price of black tea has not improved with time and is sometimes declining, despite the continued increase in the costs of production due to lack of commensurate increase in tea consumption (Owuor and Obanda, 2007).

The higher level of TF observed here with fermentation at 25 ºC seemed to have closely followed those of sensory evaluation except for brightness (Table 5). Ngure et al. (2009) reported a rapid decline in total TF at 30 ºC. A reduction of TF levels was observed with the increase of fermentation duration to 120 min. There was no significant interaction in the effects of fermentation durations and temperatures on the formation of TF. Ngure et al. (2009) showed that with the increase in fermentation duration, the TF levels decreased but TR levels increased. However, fermentation duration did not have significant effects on the TR content here. The TR significantly increased as fermentation temperature was raised. Fermentation at 30 ºC showed a marked increase in percent TR with a decline in TF. Lower TF levels with higher fermentation temperatures could be due to changes in the multiple forms of polyphenol oxidase or the transformation of TF to TR (Owuor and Obanda, 2001; Bhattacharyya et al., 2007; Ngure et al., 2009). Thus production of high quality black teas requires optimal fermentation temperature and/or duration. Cold fermentation (≤25 ºC) and/or optimum duration (60 min ≤time<90 min) are recommended for the achievement of high quality black teas (Tufekci and Guner, 1997; Owuor and Obanda, 2001). The significant correlation of total color with the TF and TR levels indicates the importance of both the biochemical components in determining total color of black tea liquor. (Obanda et al., 2004; Owuor et al., 2006). Results of other researchers showed that TRs reduced black tea brightness. These authors found that the results of spectrophotometeric measurements were in agreement with with the brightness results obtained through taster's evaluation. These results reaffirm the reports of Obanda et al. (2004) and Owuor et al. (2006) that black tea liquor brightness is reduced by high TR levels. In the production of bright black teas, cultivars need to be selected with potential

Table 1. Analysis of variance for effects of cultivar, fermentation temperature and time on the biochemical parameters in black tea.

Source	df	Mean Square			
		Theaflavin (%)	Thearubigin (%)	Total color (%)	Brightness (%)
Replication	2	0.008^{ns}	12.987^{ns}	1.251^{ns}	114.483^{ns}
Cultivar	1	0.380^{ns}	138.783^{**}	3.385^{*}	1.379^{ns}
Rep.×Cul.	2	0.072^{ns}	0.947^{ns}	0.093^{ns}	67.040^{ns}
Temperature	2	0.920^{**}	41.210^{**}	1.658^{ns}	154.872^{ns}
Time	3	0.295^{*}	3.437^{ns}	0.073^{ns}	146.815^{ns}
Cul.×Temp.	2	0.316^{ns}	0.760^{ns}	0.854^{ns}	19.682^{ns}
Cul.×Time	3	0.057^{ns}	6.015^{ns}	0.437^{ns}	25.256^{ns}
Temp.×Time	6	0.044^{ns}	7.762^{ns}	0.323^{ns}	73.375^{ns}
Cul.×Temp.×Time	6	0.079^{ns}	9.289^{ns}	0.206^{ns}	42.784^{ns}
Error	44^{y}	0.100	7.277	0.537	70.387
CV (%)		26.67	19.58	18.05	26.89

^{ns} not significant ; *,** Significant at 5% and 1% probability levels, respectively.

^{y} Error degrees of freedom for parameters of Theaflavin (%), Thearubigin (%) and Brightness (%) are '43' that had one missing value in sub-plot.

Table 2. Analysis of variance for effects of cultivar, fermentation temperature and time on the sensory parameters in black tea.

Source	df	Mean Square		
		Briskness	Brightness	Astringent
Replication	2	0.50^{ns}	0.72^{ns}	0.89^{ns}
Cultivar	1	0.88^{ns}	29.39^{ns}	1.39^{ns}
Rep.×Cul.	2	2.06^{ns}	3.39^{ns}	6.22^{*}
Temperature	2	13.17^{*}	0.72^{ns}	7.39^{*}
Time	3	6.22^{ns}	2.87^{ns}	4.20^{ns}
Cul.×Temp.	2	7.39^{ns}	2.06^{ns}	6.06^{ns}
Cul.×Time	3	1.48^{ns}	2.54^{**}	3.91^{ns}
Temp.×Time	6	3.61^{ns}	3.98^{*}	2.43^{ns}
Cul.×Temp.×Time	6	1.54^{ns}	1.76^{ns}	2.13^{ns}
Error	44	2.79	1.27	1.92
CV (%)		17.91	11.68	14.80

^{ns} not significant ,*,** Significant at 5% and 1%probability levels.

of leading to low total TR (Owuor et al., 2006). It is for this reason that in this study, we suggested cultivar "Chinese". Furthermore, the results of spectrophotometric measurements and brightness obtained from sensory evaluations showed negative, but statistically significant correlation with total color. These results showed that higher color as a result of higher TR levels are reversed with tea liquor brightness.Taster's evaluation of briskness correlated significantly with spectrophotometric measurements of total color (r = 0.331, $P\leq$ 0.01) and the R^2 of 10.96% suggests that the chemical factors responsible for results of taster's evaluation of briskness could not account for about 89.04% of the variation in spectrophotometric measurements of total color and vice versa. Muthumani and Kumar (2007) reported a balance with color and briskness in black tea liquor. Theaflavins contribute to astringency of tea liquor. There was significant positive correlation (r = 0.407, $P\leq$ 0.01) between the taster's evaluation of astringency and TF levels, and R^2 value of 16.56% suggesting that TF content alone is not a good indicator of astringency of black tea liquor. These results conformed to results of Owuor and Obanda, (1995) and those of Muthumani and Kumar, (2007) who that demonstrated the importance of another factor, termed digallate equivalent of TF, to express the astringency of black tea. Taster's evaluation of astringency correlated significantly with taster's evaluation of briskness (r = 0.417, $P\leq$ 0.01) suggesting that 17.39% (R^2) of chemical factors responsible for both quality parameters was similar, being theaflavins. Of course TF compounds contribute to the brisk and astringent taste of black tea (Yao et al., 2006).

Materials and methods

Leaf and manufacture

The leaf used in this study was plucked from the Feshalam Tea Research Center Experimental Farm, (altitude 10 m above mean sea level and latitude 37º 15′ N and longitude 49º 27′ E) in north Iran, Guilan Province. On plucking the leaf was immediately delivered to the miniature factory. The cultivars used in this study were "Promising 100" and "Chinese". The cultivars were selected to investigate their fermenting abilities. Plucking standards conformed to the normal practice of mostly two leaves and a bud, plus minor amounts of three leaves and a bud (Owuor and Obanda, 2001). Ten kg green leaf was plucked per replication. Freshly plucked tea shoots were loaded in withering trough at the rate of 5 kg m^{-2}. Ambient air was passed through the leaves for 16 h to bring about adequate physical and chemical withering. The withered leaves were passed through a mini CTC (Crush, Tear and Curl) machine four times to get adequate maceration (Muthumani and Kumar, 2007). The macerated leaf (dhool) was fermented for 30, 60, 90 and 120 min at 20, 25 and 30 ˚C. Fermentation was done in environmentally controlled units (Tea Craft, UK), and was terminated by drying in a miniature fluid bed dryer to a final moisture content of 3% (Venticell-111 Co., MMM-Group, Germany). All black tea samples were subjected to chemical analyses for TF, TR, total color and brightness measured by spectrophotometer (T80+ UV/VIS, PG Instruments Ltd, England) method, and briskness, brightness and stringency as

Table 3. The mean effect of fermentation at 20 to 30 ºC on black tea quality parameters due to variations in cultivars and fermentation time**.**

parameter	Cultivar	Fermentation time (min)				Mean
		30	60	90	120	
Theaflavin (%)	Promising 100	1.41	1.33	1.26	1.07	1.27
	Chinese	1.12	1.30	1.08	0.98	1.11
	Mean	1.27	1.32	1.17	1.03	
	HSD (P≤0.05)		0.28			0.27
	Interactions		0.47			
Thearubigin (%)	Promising 100	14.99	14.01	15.70	15.91	15.15
	Chinese	12.56	12.57	11.51	12.76	12.35
	Mean	13.78	13.29	13.61	14.34	
	HSD (P≤0.05)		2.40			0.99
	Interactions		4.04			
Total color (%)	Promising 100	4.25	4.04	4.46	4.35	4.28
	Chinese	3.91	3.93	3.60	3.92	3.84
	Mean	4.08	3.99	4.03	4.13	
	HSD (P≤0.05)		0.65			0.30
	Interactions		1.09			
Brightness (%)	Promising 100	33.69	33.00	31.06	26.60	31.09
	Chinese	30.76	34.12	33.41	27.51	31.45
	Mean	32.23	33.56	32.24	27.06	
	HSD (P≤0.05)		7.47			8.39
	Interactions		12.59			
Briskness (Taster)	Promising 100	9.89	9.67	9.22	9.00	9.44
	Chinese	9.44	10.11	9.22	8.11	9.22
	Mean	9.67	9.89	9.22	8.55	
	HSD (P≤0.05)		1.48			1.45
	Interactions		2.50			
Brightness (Taster)	Promising 100	9.67	9.00	7.89	9.44	9.00
	Chinese	10.11	11.00	10.33	9.67	10.28
	Mean	9.89	10.00	9.11	9.56	
	HSD (P≤0.05)		1.00			1.86
	Interactions		1.68			
Astringent (Taster)	Promising 100	9.44	10.11	8.78	9.67	9.50
	Chinese	9.44	9.89	9.44	8.11	9.22
	Mean	9.44	10.00	9.11	8.89	
	HSD (P≤0.05)		1.23			2.52
	Interactions		2.07			

HSD: Tukey's Studentized Range test. Briskness Key: Very Brisk-11; Brisk-9; Fairly Brisk-7; A little Brisk-5; Soft-3; Very Soft-1. Brightness Key: Very Bright-11; Bright-9; Fairly Bright-7; A little Bright-5; Dull-3; Very Dull-1. Astringent Key: Very Astringent-11; Astringent-9; fairly astringent-7; a little astringent-5; less astringent-3; Without Astringent-1.

perceived by measured by the tea experimental taster. All treatments were replicated three times. Spectrophotometric estimations were conducted to measure theaflavins (TFs), thearubigins (TRs), total color and total brightness. Two groups of pigments, the golden yellow theaflavins (TF) and the reddish brown thearubigins (TR) produced as a result of enzymatic oxidation of cathechins and their subsequent condensation during black tea manufacture, largely determine liquor characters of black tea (Ullah et al., 1984). The absorbance was measured on a UV-visible Spectrophotometer (Cintra-10, Australia). Six ml of black tea extract (100 mg/10 ml) was mixed with 6 ml of 1% (W/V) aqueous solution of anhydrous disodium hydrogen phosphate and the mixture was extracted with 10 ml of ethyl acetate by quick repeated inversion for 1 min. The separated bottom layer was drained, and the remaining the ethyl acetate layer (the TF fraction) was diluted with 5 ml ethyl acetate. Optical densities, E1, E2, and E3 were obtained on extracts prepared as follows:

1. 2.5 ml of TF extract (100mg/ 10ml) was diluted to 25 ml with methanol (E1).
2. To 0.25 ml (100mg/ 10ml) of black tea extract 2.25 ml of water was added and made up to 25 ml with methanol (E2).
3. To 0.25 ml (100mg/ 10ml) of black tea extract 0.25 ml of aqueous oxalic acid (10% W/V) and 2 ml of water was added and made up to 25 ml with methanol (E3). Optical densities of E1, E2 and E3 were measured at 380 and 460 nm.

At 380 nm %TF= 2.3 × E3
%TR= 7.06 (4E3-E1)
At 460 nm total color= 6.25 × 4E2
% total brightness= E1/4E2× 100.

Ranking of liquor briskness, brightness and astringecyt by taster

Randomly numbered black tea samples were subjected to test by a professional taster for briskness, brightness and astringent ranking. The following scales were used to rank the levels of these parameters in the tea liquor; very brisk-11;

Table 4. The mean effect of fermentation time of 30 to 120 minutes on black tea quality parameters due to variations in cultivars and fermentation temperature.

parameter	Cultivar	Fermentation temperatures (ºC)			Mean
		20	25	30	
Theaflavin (%)	Promising 100	1.34	1.39	1.07	1.27
	Chinese	0.94	1.44	0.98	1.12
	Mean	1.14	1.42	1.03	
	HSD ($P\leq 0.05$)		0.22		0.27
	Interactions		0.38		
Thearubigin (%)	Promising 100	13.72	15.66	16.07	15.15
	Chinese	10.88	12.54	13.64	12.35
	Mean	12.30	14.10	14.86	
	HSD ($P\leq 0.05$)		1.89		0.99
	Interactions		3.28		
Total color (%)	Promising 100	4.28	4.40	4.08	4.25
	Chinese	3.41	4.23	3.88	3.84
	Mean	3.85	4.32	3.98	
	HSD ($P\leq 0.05$)		0.51		0.30
	Interactions		0.89		
Brightness (%)	Promising 100	33.25	31.54	28.47	31.09
	Chinese	32.11	34.16	28.08	31.45
	Mean	32.68	32.85	28.28	
	HSD ($P\leq 0.05$)		5.87		8.36
	Interactions		10.21		
Briskness (Taster)	Promising 100	9.83	10.00	8.50	9.44
	Chinese	8.33	10.33	9.00	9.22
	Mean	9.08	10.17	8.75	
	HSD ($P\leq 0.05$)		1.17		1.45
	Interactions		2.03		
Brightness (Taster)	Promising 100	9.17	8.67	9.17	9.00
	Chinese	10.50	10.50	9.83	10.28
	Mean	9.83	9.58	9.50	
	HSD ($P\leq 0.05$)		0.78		1.86
	Interactions		1.36		
Astringent (Taster)	Promising 100	9.67	9.67	9.17	9.50
	Chinese	8.33	10.33	9.00	9.22
	Mean	9.00	10.00	9.08	
	HSD ($P\leq 0.05$)		0.97		2.52
	Interactions		1.68		

HSD: Tukey's Studentized Range test. Briskness Key: Very Brisk-11; Brisk-9; Fairly Brisk-7; A little Brisk-5; Soft-3; Very Soft-1. Brightness Key: Very Bright-11; Bright-9; Fairly Bright-7; A little Bright-5; Dull-3; Very Dull-1. Astringent Key: Very Astringent-11; Astringent-9; fairly astringent-7; a little astringent-5; less astringent-3; Without Astringent-1.

brisk-9; fairly brisk-7; a little brisk-5; soft-3; very soft-1 (Obanda et al., 2001); very bright-11; bright-9; fairly bright-7; a little bright-5; dull-3; very dull-1 (Obanda et al., 2001; Obanda et al., 2004); very astringent-11; astringent-9; fairly astringent-7; a little astringent-5; less astringent-3; without astringent-1 (Owuor and Obanda, 1995).

Reagents

Disodium hydrogen phosphate, ethyl acetate and methanol came from Merck (Darmstadt, Germany). Oxalic acid from Sigma-Aldrich (St. Louis, Mo, USA) and the rest of the solvents and reagents were of analytical grade while water was doubled distilled.

Sensory evaluation

An experienced professional tea tester at the Tea Research Center in Lahijian evaluated the black teas. The testers have expert knowledge of black teas, which he auctions regularly.

Statistical analysis

The results were analyzed of variance using (SAS version 9.1) as split plot factorial with cultivars as main treatments and fermentation temperature and duration on sub-plots, in pilot randomized complete block design (RCBD) with 3 replications. The analytical procedures used to demonstrate relationships among chemical substances on the black tea quality were correlation and regression analyses. A simple correlation indicated the strength of the linear relationship between two variables. The correlation procedure was administered on taster's scores and spectrophotometric measurements of black tea quality parameters with SAS version 9.1 (SAS, 1985). Regression analysis was used to determine the major substances contributing to brightness and total color in the spectrophotometric measurements and to briskness, brightness and astringency in taster's scores. The independent explanatory variables were TR and TF. The software used for analysis was SPSS/PC^{+} "Stepwise" (version 14.0).

Table 5. The mean effect on black tea quality parameters of two cultivars due to variations in fermentation temperature and time.

Parameter	Temperature (ºC)	Fermentation time (min)				Mean
		30	60	90	120	
Theaflavin (%)	20	1.14	1.20	1.15	1.07	1.14
	25	1.60	1.51	1.35	1.21	1.42
	30	1.07	1.23	1.01	0.78	1.02
	Mean	1.27	1.31	1.17	1.02	
	HSD ($P \leq 0.05$)		0.28			0.22
	Interactions		0.63			
Thearubigin (%)	20	12.41	13.13	11.21	12.45	12.30
	25	13.49	13.97	14.64	14.53	14.16
	30	15.69	12.76	14.97	16.01	14.86
	Mean	13.86	13.29	13.61	14.33	
	HSD ($P \leq 0.05$)		2.40			1.89
	Interactions		5.37			
Total color (%)	20	3.82	3.85	3.65	4.06	3.85
	25	4.28	4.47	4.51	4.15	4.35
	30	4.15	3.64	3.93	4.19	3.98
	Mean	4.08	3.99	4.03	4.13	
	HSD ($P \leq 0.05$)		0.65			0.51
	Interactions		1.45			
Brightness (%)	20	32.36	33.64	34.48	30.24	32.68
	25	37.74	33.25	30.11	30.51	32.90
	30	26.78	33.79	32.12	20.42	28.28
	Mean	32.29	33.56	32.24	27.06	
	HSD ($P \leq 0.05$)		7.47			5.87
	Interactions		16.72			
Briskness (Taster)	20	8.67	9.33	9.00	9.33	9.08
	25	11.00	10.33	10.00	9.33	10.17
	30	9.33	10.00	8.67	7.00	8.75
	Mean	9.67	9.89	9.22	8.56	
	HSD ($P \leq 0.05$)		1.48			1.17
	Interactions		3.32			
Brightness (Taster)	20	10.33	10.33	10.00	8.67	9.83
	25	9.67	9.33	8.67	10.67	9.58
	30	9.67	10.33	8.67	9.33	9.50
	Mean	9.89	10.00	9.11	9.56	
	HSD ($P \leq 0.05$)		1.00			0.78
	Interactions		2.24			
Astringent (Taster)	20	8.67	9.67	8.33	9.33	9.00
	25	10.00	10.33	10.00	9.67	10.00
	30	9.67	10.00	9.00	7.67	9.08
	Mean	9.44	10.00	9.11	8.89	
	HSD ($P \leq 0.05$)		1.23			0.97
	Interactions		2.75			

HSD: Tukey's Studentized Range test. Briskness Key: Very Brisk-11; Brisk-9; Fairly Brisk-7; A little Brisk-5; Soft-3; Very Soft-1.Brightness Key: Very Bright-11; Bright-9; Fairly Bright-7; A little Bright-5; Dull-3; Very Dull-1. Astringent Key: Very Astringent-11; Astringent-9; fairly astringent-7; a little astringent-5; less astringent-3; Without Astringent-1.

Conclusion

Theaflavins may have considerable health benefits for human beings. Thus, aside from their influence on liquor astringency and briskness, these results highlight the possibility of manipulating processing conditions of duration and temperature to enhance the health benefit potential of black tea. Maintaining medium fermentation temperature (25 ºC) and short duration (60 min) will ensure greater formation of TFs. The resultant black teas are then brisk, bright, and astringent and probably offer more benefit to human health. A better prediction of liquor brightness and total color of black tea will be achieved with inclusion of TF and TR than could be obtained with TF or TR alone. Briskness and astringency were associated with TF than with TR. Therefore, they were best predicted with TF. Evaluation of brightness by taster was associated to TR, and was best predicted with TR. Variations in fermentation duration were used to achieve maximum levels of different black tea parameters in cultivars. Cultivar "Promising 100" produced higher level of TR and more color as compared to the cultivar "Chinese". Higher TR content and total color developed by medium fermentation temperature (25°C) and short

Table 6. The mean effect of fermentation on black tea quality due to variations in cultivars and fermentation temperature and time.

Parameter	Cultivar	Fermentation temperature (ºC)	Fermentation time (min)				Mean
			30	60	90	120	
Theaflavin (%)	Promising 100	20	1.41	1.37	1.25	1.33	1.34
		25	1.66	1.32	1.50	1.06	1.39
		30	1.16	1.29	1.03	0.81	1.07
	Chinese	20	0.87	1.04	1.05	0.81	0.94
		25	1.51	1.70	1.19	1.36	1.44
		30	0.99	1.17	1.00	0.75	0.98
	Mean		1.27	1.32	1.17	1.03	
	HSD ($P \leq 0.05$)			0.28			
	Interactions			1.00			0.38
Thearubigin (%)	Promising 100	20	14.22	11.87	13.72	15.08	13.72
		25	14.48	15.26	16.83	16.06	15.66
		30	16.27	14.88	16.55	16.58	16.07
	Chinese	20	10.59	14.39	8.70	9.83	10.88
		25	11.99	12.68	12.45	13.01	12.54
		30	15.11	10.63	13.39	15.44	13.64
	Mean		13.78	13.29	13.61	14.34	
	HSD ($P \leq 0.05$)			2.40			
	Interactions			8.54			3.28
Total color (%)	Promising 100	20	4.03	4.05	4.26	4.78	4.28
		25	4.42	4.41	4.94	4.11	4.47
		30	4.31	3.68	4.18	4.15	4.08
	Chinese	20	3.61	3.65	3.05	3.34	3.41
		25	4.13	4.54	4.08	4.19	4.23
		30	4.00	3.60	3.68	4.24	3.88
	Mean		4.08	3.99	4.03	4.13	
	HSD ($P \leq 0.05$)			0.65			
	Interactions			2.31			0.89
Brightness (%)	Promising 100	20	35.63	36.08	29.93	31.37	33.25
		25	38.63	30.20	30.01	27.32	31.54
		30	26.80	32.71	33.25	21.12	28.47
	Chinese	20	29.09	31.20	39.03	29.11	32.11
		25	36.41	36.29	30.21	33.71	34.16
		30	26.76	34.86	30.99	19.72	28.08
Brightness (%)	Mean		32.23	33.56	32.24	27.06	
	HSD ($P \leq 0.05$)			7.47			
	Interactions			26.57			10.21
Briskness (Taster)	Promising 100	20	9.67	9.67	9.00	11.00	9.83
		25	11.00	9.67	10.33	9.00	10.00
		30	9.00	9.67	8.33	7.00	8.50
	Chinese	20	7.67	9.00	9.00	7.67	8.33
		25	11.00	11.00	9.67	9.67	10.33
		30	9.67	10.33	9.00	7.00	9.00
	Mean		9.67	9.89	9.22	8.55	
	HSD ($P \leq 0.05$)			1.48			
	Interactions			5.28			2.03
Brightness (Taster)	Promising 100	20	10.33	9.67	9.00	7.67	9.17
		25	9.00	7.67	7.00	11.00	8.67
		30	9.67	9.67	7.67	9.67	9.17
	Chinese	20	10.33	11.00	11.00	9.67	10.50
		25	10.33	11.00	10.33	10.33	10.50
		30	9.67	11.00	9.67	9.00	9.83
	Mean		9.89	10.00	9.11	9.56	
	HSD ($P \leq 0.05$)			1.00			
	Interactions			3.56			1.36
Astringent (Taster)	Promising 100	20	9.67	10.33	7.67	11.00	9.67
		25	9.67	9.67	9.67	9.67	9.67
		30	9.00	10.33	9.00	8.33	9.17
	Chinese	20	7.67	9.00		7.67	8.33
		25	10.33	11.00	9:00	9.67	10.33
		30	10.33	9.67	9.00	7.00	9.00
	Mean		9.44	10.00	9.11	8.89	
	HSD ($P \leq 0.05$)			1.23			
	Interactions			4.38			1.68

HSD: Tukey's Studentized Range test. Briskness Key: Very Brisk-11; Brisk-9; Fairly Brisk-7; A little Brisk-5; Soft-3; Very Soft-1. Brightness Key: Very Bright-11; Bright-9; Fairly Bright-7; A little Bright-5; Dull-3; Very Dull-1. Astringent Key: Very Astringent-11; Astringent-9; fairly astringent-7; a little astringent-5; less astringent-3; Without Astringent-1.

Table 7. Correlation coefficients (r) between Theaflavin%, thearubigin%, total color%, brightness%, briskness, brightness and astringent.

Parameter	Theaflavin (%)	Thearubigin (%)	Total color (%)	Brightness (%)	Briskness[y]	Brightness[y]	Astringent[y]
Theaflavin (%)	1.000	0.041	0.385**	-0.193	0.863**	-0.193	0.407**
Thearubigin (%)	0.041	1.000	0.704**	-0.401**	0.001	-0.401**	0.029
Total color (%)	0.385**	0.704**	1.000	-0.498**	0.331**	-0.498**	0.198
Brightness (%)	-0.193	-0.401**	-0.498**	1.000	-0.209	1.000	0.115
Briskness[y]	0.863**	0.001	0.331**	-0.209	1.000	-0.209	0.417**
Brightness[y]	-0.193	-0.401**	-0.498**	1.000	-0.209	1.000	0.115
Astringent[y]	0.407**	0.029	0.198	0.115	0.417**	0.115	1.000

[y] Sensory parameters that tested by taster.
**Correlation is significant at the 1% level (two-tailed).

fermentation duration (60 min). Thus medium fermentation temperature and short temperature favour production of thicker and darker colored black tea. Maximum sensory evaluation scores, briskness, brightness and astringent levels at different fermentation temperatures were attained at different fermentation durations. At low fermentation temperature maximum sensory evaluation scores were obtained. Thus production of high quality black teas at lower fermentation temperature requires longer fermentation duration, and maintenance of low fermentation temperature, though requiring longer fermentation duration, ensures that the resultant black teas are of better quality. There was significant ($P \leq 0.05$) interaction between fermentation duration and temperature in parameters of black tea cultivars indicating that the rate and patterns of their development varied with time at different temperature. Thus it is not easy to extrapolate optimal fermentation duration when there is change in temperature. It is therefore important that optimal fermentation duration is established for every cultivar at different temperatures for production of high quality black teas.

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